Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, No. 5, pp. 449–452, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00

Short Communication

Separation of chloroquine enantiomers by high-performance liquid chromatography

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Keywords: *High-performance liquid chromatography; chloroquine; enantiomer separation; α_1-acid glycoprotein.*

Introduction

Chloroquine and other antimalarial drugs have been in use for over three decades. The drug is available as a racemic mixture and is used as such in the various pharmaceutical dosage forms. Literature surveys have revealed very little regarding the chiral resolution of chloroquine. Riegel and Sherwood [1] reported that enantiomorphs of chloroquine, chemically resolved using D-bromocamphorsulphonic acid, showed no significant differences, either as regards activity in birds or toxicity in dogs, from that of the racemate. However, the authors admitted that the crystals they obtained were hygrosopic and somewhat unstable. Moreover, only the specific rotation, $[\alpha]_D^{25}$, was taken as a measure of enantiomeric purity and even then the results obtained for each enantiomer were not consistent.

Recently, increasing interest has been directed towards the separation of drug enantiomers by high-performance liquid chromatography (HPLC) [2–6]. The technique can provide a quick, reliable and sensitive method for chiral resolution and for the analysis of the individual enantiomers. In principle, therefore, HPLC can be used for studies on the selective activities and toxicity of enantiomers in clinical studies and for chiral purity assessment in quality control.

The aim of the present work was to develop an HPLC procedure for the separation of chloroquine enantiomers, based on α_1-acid glycoprotein as the chiral stationary phase. The effects of the principal operational parameters (concentration of organic modifier, pH, molarity of sodium chloride and buffer molarity) have been systematically characterized. The applicability of the method for quantitative analysis of the enantiomers is also demonstrated.

Experimental

Apparatus

A first-generation Enantiopak column (LKB Produkter, Bromma, Sweden) (100 × 4 mm i.d.) containing α_1-acid glycoprotein (AGP) immobilized on 10-μm diethylaminoethyl silica gel was used. Chromatography was performed using an LKB model 2150 pump, a Pye Unicam PU 4025 UV detector set at 254 nm and a Perkin Elmer recorder. All pH values were determined using a Pye pH meter.

Procedures

The optimized mobile phase employed was propan-2-ol–phosphate buffer (10 mM, with 0.1 M sodium chloride) (4:96, v/v) at pH 6.75 (unless otherwise stated). The mobile phase was degassed by sonication immediately before use. All chromatograms were run at room temperature with a flow rate of 0.3 ml min^{-1}.

Materials

Chloroquine diphosphate was kindly donated by Wellcome Laboratories (Dartford,

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UK). Analytical grade sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride were used as received. The buffer was prepared by dissolving appropriate amounts in doubly distilled water. Analytical grade propan-2-ol was used as the organic modifier.

Preparation of standard curve

A stock solution of chloroquine diphosphate (1 mg ml^{-1}) in phosphate buffer was prepared. Dilutions were made to obtain a series of solutions with concentrations ranging from 20 to 500 μg ml^{-1}. Duplicate injections (10 μl each) were made for each solution and the mean peak height calculated and plotted against the corresponding concentration. Relative standard deviations were calculated from the results obtained by repeated injections (n = 5) from each of two solutions, one containing 50 μg ml^{-1} and the other 200 μg ml^{-1}.

Results and Discussion

Effect of the concentration of propan-2-ol on k′ *and on the resolution of chloroquine enantiomers*

A series of mobile phases of phosphate buffer (10 mM; pH 6.75) containing 0.1 M sodium chloride and different concentrations (2–10% v/v) of propan-2-ol were prepared. Duplicate injections (5 μl each) of chloroquine diphosphate in phosphate buffer (200 μg ml^{-1}) were examined. The results are plotted in Fig. 1. It can be seen that the best resolution (R_s = 1.15) was achieved with a concentration of 4% propan-2-ol. The number of theoretical plates per metre was found to be 6.4 × 10^3 and 6.2 × 10^3 for the first and second enantiomer, respectively. It appears that the efficiency of Enantiopak chiral columns is low, compared with conventional reversed-phase columns. However, a fairly good separation was obtained working with suitable efficiency using propan-2-ol at this concentration.

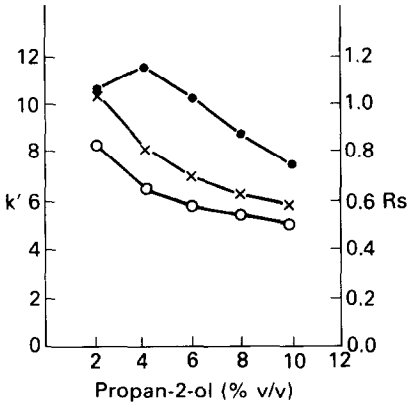

Figure 1
Effect of changes in propan-2-ol concentration on capacity factor (k') and resolution (R_s) data for chloroquine enantiomers separated on an AGP column with mobile phase: phosphate buffer–propan-2-ol (pH 6.75). Key to symbols: ○, first eluting enantiomer; ×, second enantiomer; ●, resolution data.

Effect of pH on the chromatography of chloroquine enantiomers

Mobile phases of phosphate buffer (10 mM), 0.1 M sodium chloride, propan-2-ol (4%) all variations in pH were used in this study to determine the effect of pH on the observed chromatographic parameters of chloroquine enantiomers. It is evident from Fig. 2 that the pH factor is critical in the resolution of racemic chloroquine. At low pH values (4–6) no resolution could be achieved. Optimum column efficiency (6.3 × 10^3 theoretical plates per metre) was observed at a pH value of 6.75. Lower or higher pH values appeared to decrease the efficiency of the column to a great extent. The resolution increased progressively with pH increase, being 1.15 at pH 6.75. This was considered to be adequate for further work. Further increase in pH would have

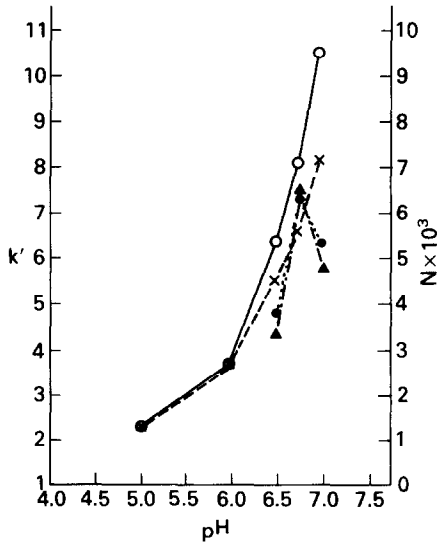

Figure 2
The k' and column efficiency (N, plates per metre) data for chloroquine enantiomers and their relationship to pH for a mobile phase containing 4% v/v propan-2-ol. Key to symbols: ×, k' for first enantiomer; ○, k' for second enantiomer; ●, N for first enantiomer; ▲, N for second enantiomer.

damaged the column, according to the manufacturer's instructions.

Effect of changing the concentration of sodium chloride and the buffer molarity

Little effect, on retention time, column performance or resolution, was observed when the concentration of phosphate buffer was adjusted to 0.05, 0.1 or 0.2 M. However, with different sodium chloride concentrations the effect was much more significant. Increase in the concentration of sodium chloride resulted in shorter retention times, but did not seem to alter the resolution of the enantiomers (Fig. 3). In the absence of sodium chloride, the drug took a long time (*ca* 1 h) to elute and the peaks obtained were then too broad to be measured. With the incorporation of as little as 0.05 M sodium chloride, there was a remarkable shortening in retention time and peaks of reasonable efficiency were then obtained in about 20–25 min (Fig. 4).

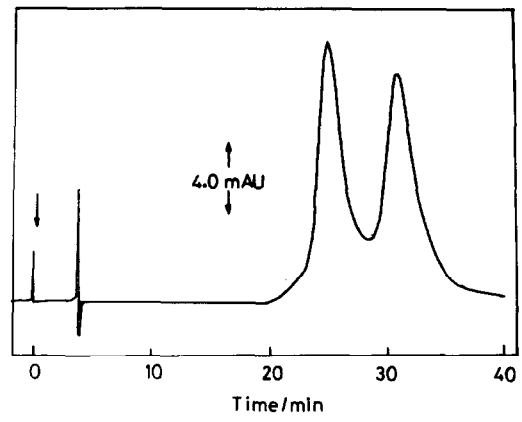

Figure 4
Separation of chloroquine enantiomers on a first-generation AGP column; concentration 200 μg ml^{-1}; injection volume, 5 μl. For chromatographic conditions, see text.

Quantitative figures of merit

Mean peak heights for each enantiomer from duplicate injections of solutions of chloroquine with five concentrations ranging from 20–500 μg ml^{-1} were plotted against the corresponding concentrations. Linear relationships were obtained for each enantiomer of chloroquine. The regression equations obtained at a sensitivity of 40 mAU were:

$$y = 0.0576x - 0.573 \text{ (first enantiomer);}$$
$$y = 0.048x - 0.264 \text{ (retarded enantiomer).}$$

The relative standard deviation (n = 5) at concentrations of 200 μg ml^{-1} and 50 μg ml^{-1} were 1.2 and 0.9%, respectively, for the first enantiomer, and 1.3 and 1.0% for the retarded enantiomer. The sensitivity of the method in terms of ng of each enantiomer injected on column for SNR = 2, was *ca* 80 ng.

The present work reports the enantiomeric separation of chloroquine enantiomers by a quick, simple and sensitive HPLC procedure. The method is suitable for the quantitative estimation of these enantiomers. It can be considered as a suitable starting point for the isolation of very small amounts of the enantiomers. Thus it could provide a reliable and less tedious alternative to the chemical isolation of chloroquine enantiomers.

Hermansson *et al.* [7] have reported a similar method for the chiral determination of chloroquine enantiomers in plasma and urine. Although the report did not include a comprehensive analytical assessment of the method employed, or any figures of merit for its quantitative performance, the authors did observe evidence for the stereoselective metabolism of this drug. It is the intention of the present work to define a rapid, sensitive and reliable assay procedure for the analysis of the individual chloroquine enantiomers, suitable both for bioanalytical studies in chiral metabolism and for chiral quality control on dosage forms.

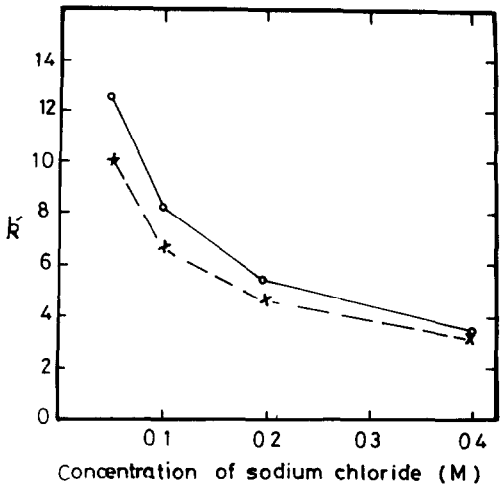

Figure 3
Effect of sodium chloride concentration on k' for chloroquine enantiomers with a mobile phase comprising phosphate buffer (pH 6.75) and propan-2-ol (96:4, v/v). Key to symbols: ×, first eluting enantiomer; ○, second enantiomer.

The present method should in principle be practicable using the recently developed second generation AGP column ("Chiral–AGP", Chrom Tech AB, Sweden), with appropriate modification of the eluent parameters [8].

Acknowledgements — This investigation was made possible through a British Council link scheme between the Departments of Pharmaceutical Chemistry in the Universities of Bradford and Khartoum. Dr Terry Noctor is thanked for valuable discussions and advice.

References

[1] B. Riegel and L.T. Sherwood Jr, *J. Am. Chem. Soc.* **71**, 1129–1130 (1949).
[2] D.W. Armstrong, J. Ward, R.D. Armstrong and T.E. Beesley, *Science* **232**, 1132–1135 (1986).
[3] D.W. Armstrong and W. DeMond, *J. Chromatogr. Sci.* **22**, 411–415 (1984).
[4] J. Hermansson, *J. Chromatogr.* **269**, 71–80 (1983).
[5] J. Hermansson and M. Eriksson, *J. Liq. Chromatogr.* **9**, 621–639 (1986).
[6] T.A.G. Noctor, B.J. Clark and A.F. Fell, *Anal. Proc.* **23**, 441–443 (1986).
[7] D. Ofori-Adjei, O. Ericsson, B. Lindstrom, J. Hermansson, K. Adjepon-Yamoah and F. Sjoqvist, *Therap. Drug Monit.* **8**, 457–461 (1986).
[8] K. Ibrahim (work in preparation).

[*Received for review 28 March 1990; revised manuscript received 11 May 1990*]